Epidermolysis Bullosa Simplex: Recurrent and *De Novo* Mutations in the KRT5 and KRT14 Genes, Phenotype/Genotype Correlations, and Implications for Genetic Counseling and Prenatal Diagnosis

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Epidermolysis bullosa simplex (EBS) is a mechano-bullous disorder characterized by intraepidermal blistering within the basal keratinocytes as a result of trauma to the skin. As part of the DNA diagnostics program, our laboratory has analyzed a cohort of 57 patients with the initial referral diagnosis of EBS. Among these patients, 18 were found to harbor heterozygous mutations in the keratin 5 or keratin 14 genes, KRT5 and KRT14, respectively, whereas in 14 cases, the disease was associated with mutations in both alleles of the plectin gene. Among the keratin mutations, 12 were distinct and six were novel, and in most cases there was no family history of a blistering disease. Prenatal diagnosis of eight pregnancies with keratin gene mutations, at risk for EBS either because one of the parents was affected (three cases) or history of a previously affected child as a result of a *de novo* mutation (five cases), predicted two fetuses being affected and six being normal. No recurrence of the *de novo* mutations in these pregnancies was disclosed. Collectively, the data suggest that a significant number of cases diagnosed as EBS are due to plectin mutations, and many cases result from *de novo* mutations in KRT5 and KRT14 genes. These findings have implications for genetic counseling and prenatal diagnosis for EBS.

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Epidermolysis bullosa (EB), a group of heritable mechanobullous disorders, displays a spectrum of severity of skin manifestations (Fine *et al*, 1999). EB is traditionally divided into three broad categories based on the level of tissue separation within the cutaneous basement membrane zone (BMZ), as determined by diagnostic transmission electron microscopy and/or immunoepitope mapping (Fine *et al*, 2000). One of them is EB simplex (EBS), which is characterized by tissue separation within the basal layer of epidermis due to fragility of basal keratinocytes. In junctional forms of EB tissue separation takes place within the dermo-epidermal basement membrane, primarily at the level of lamina lucida, whereas in dystrophic forms the blistering occurs below the lamina densa within the upper papillary dermis at the level of anchoring fibrils (Uitto and Richard, 2004). The variability in the phenotypic presentation of EB is now known to reflect the presence of mutations in ten different genes (Pulkkinen *et al*, 2002; Uitto and Richard, 2004). The compartmentalized expression of the affected genes within the cutaneous BMZ and in extracutaneous tissues, as well as the types and positions of the mutations and their consequences at the mRNA and protein levels, explain the tremendous variability in EB phenotype (Uitto and Pulkkinen, 2001).

Abbreviations: BMZ, basement membrane zone; EBS, epidermolysis bullosa simplex

EBS is the most common subtype of EB, and epidemiological data emanating from the US National Epidermolysis Bullosa Registry have suggested that EBS accounts for at least half of all cases of EB (Fine *et al*, 1999). Furthermore, the relative incidence of EBS may be much higher than that since a proportion of affected patients, most notably those with mild blistering, do not come to the attention of physicians. Traditionally, EBS has been divided into subcategories reflecting the clinical severity and the types and distribution of the lesions. EBS Dowling–Meara, which presents with generalized herpetiform groups of vesicles or bullae, can be associated with early infant mortality (Dowling and Meara, 1954). EBS Köbner manifests with generalized blistering (Köbner, 1886), whereas EBS Weber–Cockayne presents with localized blistering primarily on the hands and feet (Cockayne, 1938). A rare subtype of EBS is associated with mottled pigmentation (Fischer and Gedde-Dahl, 1979). These four types of EBS are inherited in most cases in an autosomal-dominant fashion. An autosomal-recessive form of EB associated with late-onset muscular dystrophy (EBS-MD) has been classified as EBS because the blistering is intracellular within the basal keratinocytes. Similarly, a rare autosomal-dominant variant of EBS, the so-called Ogna type, harbors mutations in the plectin gene (Koss-Harness *et al*, 2002). It should be noted, however, that blistering in EBS-MD and EBS-Ogna is low at the level of hemidesmosomes at the apical pole of keratinocytes (see Uitto *et al*, 1996; Pfendner *et al*, 2005).

In the classic forms of EBS, the basal cells disintegrate as a result of shearing trauma to the skin, and the cell fragility is due to defective intermediate keratin filament network resulting from mutations in the basal keratin genes, KRT5 and KRT14 (Irvine and McLean, 1999; Cassidy *et al*, 2002; Rugg and Leigh, 2004). The majority of the cases show autosomal-dominant inheritance, although rare autosomal-recessive cases have been described (Hovnanian *et al*, 1993; Batta *et al*, 2000). In addition, a number of cases are sporadic with no family history of blistering diseases.

The DebRA Molecular Diagnostics Laboratory, located at Jefferson Medical College, Philadelphia, Pennsylvania, has provided DNA-based diagnostic services to the global EB community since 1996. As of today, we have analyzed over 1000 families with different forms of EB, most with severe recessive dystrophic or lethal junctional variants. Among the families studied, there were 57 individuals referred to us with the diagnosis of EBS. DNA analysis revealed specific mutations either in KRT5 or KRT14 in 18 families (see below). Careful examination of the remaining 39 cases reveals that in 16 cases, the referral diagnosis of EBS was not correct or was not consistent with electron microscopic or immunofluorescence findings, whereas 14 cases were found to harbor mutations in PLEC1 (see Pfendner *et al*, 2005). These observations reinforce the importance of good histopathological, immunofluorescence, and ultrastructural data prior to molecular genetic screening. In the remaining nine cases, the electron microscopic and/or immunohistochemical data supported the diagnosis of EBS, but no mutations in the KRT5, KRT14, or plectin genes could be identified in spite of extensive sequencing of the exons and flanking introns. In the latter cases, it is possible that mutations in these three candidate genes may exist in the regions not examined by us for mutations, including the promoter regions and intronic sequences beyond 100–200 bp away from the intron–exon junctions.

DNA was isolated from peripheral blood specimens, or in case of the prenatal testing, from chorionic villus samples, after a written informed consent was obtained from the patients or their guardians. The studies were approved by the Institutional Review Board of Thomas Jefferson University, and they adhere to the Declaration of Helsinki Principles. PCR amplification of exons and flanking intronic sequences of KRT5 and KRT14 (Whittock *et al*, 2000; Wood *et al*, 2003), followed by direct dideoxide nucleotide sequencing, of the probands' DNA resulted in identification of heterozygous keratin 5 or keratin 14 gene mutations in 18 families (Table I). Twelve distinct mutations were disclosed, six of them previously unpublished. In seven cases, the mutations resided in KRT5 and in eleven cases in KRT14, indicating that mutations in either gene can result in EBS at an approximately equal frequency. In 11 cases, DNA was available from both parents, and in only three cases the corresponding mutation was present in the peripheral blood DNA of one of the parents (Table I). In seven families in which DNA samples were not available from the parents, there was no family history of a blistering skin disease, and specifically, the parents were clinically unaffected. Based on this information, it appears that a large number of cases in this cohort (15 of 18) may represent *de novo* mutations in KRT5 or KRT14. It is likely, however, that more severe cases and in particular those who have no known family history are overrepresented in this cohort, as these cases were referred to for genetic evaluation at birth or shortly thereafter to clarify the mode of inheritance.

Examination of the mutation database indicated that, with the exception of one case (no. 16), the mutations were missense substitutions primarily within the helix initiation or termination peptides. A recurrent R125C mutation was encountered in three families, and the same arginine residue was also mutated twice to histidine (R125H). The previously reported mutation N123S was disclosed in four families. Careful examination of the clinical features, as reported by the referring physicians and summarized in Table I, revealed that the recurrent mutation N123S appears to be associated with severe generalized blistering with oral mucous membrane involvement (EBS Dowling–Meara). In one case (no. 1), the severity of the airway involvement necessitated tracheotomy. One case of EBS with palmoplantar keratoderma and mottled pigmentation harbored the P25L mutation in KRT5 (no. 13), confirming previously reported association of this particular mutation with the mottled pigmentation phenotype (Uttam *et al*, 1996; Nobuhara, 2003). Finally, a single amino acid deletion (dell183) (no. 16), a *de novo* event, resulted in generalized blistering but without scarring (EBS Köbner).

EBS has been considered to be a relatively mild disease; however, our observations clearly indicate that EBS can be severe. The more severe phenotype appears to be associated with certain mutations, such as N123S of KRT14. This mutation resides within the 1A domain of the keratin molecule and is predicted to severely perturb the intermediate filament network.

These observations clearly impact the genetic counseling of the families regarding the recurrence risk of EBS to clinically normal parents with a previously affected child with EBS. The extent of the risk for recurrence in each family is variable, but estimates from other heritable diseases suggest a range from 0.02% to 14% depending on the disorder (Byers *et al*, 1988; Bakker *et al*, 1989; Green *et al*, 1999; Mettler and Fraser, 2000). The *a priori* risk can be determined from a general formula, in which there is only one affected sibling in a family of otherwise untyped normal siblings as between 1.6% and 4.8% (van der Meulen *et al*, 1995). For genetic counseling purposes, we quote an EBS recurrence risk in a family of one affected offspring as between 2% and 5%, consistent with the calculated value. In case of EB, the risk of recurrence is real, as has been documented by *de novo* cases with dystrophic or junctional EB (Cserhalmi-Friedman *et al*, 2001, 2002).

As indicated above, the severity of EBS is highly variable, but at one end of the spectrum, the disease can result, although rarely, in premature demise of the affected individuals during the early postnatal period. In such cases, prenatal diagnosis has been requested by the parents in subsequent pregnancies (Rugg *et al*, 2000; Pfendner *et al*, 2003). We have performed DNA-based prenatal diagnosis in eight EBS families with established keratin mutations (Table II). In three cases (S1, S7, and S8), one of the parents was affected with EBS, whereas in the remaining five cases the parents were clinically normal but they had a previously affected child with a *de novo* keratin mutation. Among the

Table I. Mutations in KRT5 or KRT14 and phenotypes of families with epidermolysis bullosa simplex

Patient number	Gene	Mutation[a]	Keratin domain of mutation[b]	Inheritance	Parental mutation[c], phenotype	Clinical features	EM/immunofluorescence findings
1	KRT14	N123S	1A	*De novo*	−/−, unaffected	Severe widespread blistering, airway involvement, tracheotomy G tube	Consistent with EBS
2	KRT5	E168L	H1	Paternal	+/−, father affected	Generalized blisters, aplasia cutis congenita at birth	Keratinocyte cleavage plane
3	KRT5	**I467M**	2B	*De novo*	−/−, unaffected	Generalized blisters, palmoplantar hyperkeratosis	Keratinocyte cleavage plane
4	KRT14	R125C	1A	*De novo*	N/A, no family history	Generalized blistering at birth	Keratinocyte cleavage consistent with EBS
5	KRT14	R125H	1A	*De novo*	N/A, no family history	Severe blistering, oral erosions	Not available
6	KRT5	**I183F**	1A	*De novo*	−/−, unaffected	Severe generalized blistering, G tube	Keratinocyte cleavage plane
7	KRT14	R125C	1A	*De novo*	−/−, unaffected	Generalized blistering	Cleavage plane in basilar layer
8	KRT14	R125C	1A	*De novo*	−/−, unaffected	Generalized blistering	Intraepidermal cleavage
9	KRT5	**E477K**	2B	*De novo*	−/−, unaffected	Severe generalized blistering	Intraepidermal cleavage, clumping of tonofilaments
10	KRT5	**I161S**	H1	Maternal	−/+, mother affected	Blistering of hands and feet	Split in basal cell layer
11	KRT14	M119T	1A	*De novo*	N/A, no family history	Severe blistering, palmoplantar hyperkeratosis	Unknown
12	KRT14	N123S	1A	*De novo*	N/A, no family history	Severe blistering on trunk, extremities, oral, and esophageal mucosa	Cleavage in basal cell layer
13	KRT5	P25L	V1	Paternal	+/−, father affected	Blistering, palmoplantar keratoderma, mottled pigmentation	Not done
14	KRT14	**Q120P**	1A	*De novo*	−/−, unaffected	Generalized blistering	Cytokeratin staining on both sides of cleavage plane
15	KRT14	N123S	1A	*De novo*	N/A, no family history	Generalized blistering	Cytokeratin staining on both sides of cleavage plane
16	KRT5	**Del I183**	1A	*De novo*	−/−, unaffected	Generalized blistering without scarring	Cleavage plane in basal cell layer
17	KRT14	R125H	1A	*De novo*	N/A, no family history	Generalized blistering	Cleavage plane in keratinocyte layer
18	KRT14	N123S	1A	*De novo*	N/A, no family history	Severe generalized blistering	Cytokeratin staining on both sides of cleavage plane. Cleavage plane in basal cell layer

[a]Mutations not previously reported are in bold type.
[b]Domain assignments as given on http://www.interfil.org/IFstructure/type2/k5.htm or http://www.interfil.org/IFstructure/type1/k14st.htm
[c]N/A, parental DNA not available.
EM, electron microscopy.

Table II. Prenatal diagnosis for epidermolysis bullosa (EB) simplex

Family number[a]	Test no.	Test no. in the same family	Affected family member [b]	EB subtype[c]	Gene	Mutation	Predicted outcome	Confirmation[d]
4	S1	1	Father	EBS	KRT14	R125C	Affected	Affected
5	S2	1	Sibling	EBS	KRT14	R125H	Normal	Normal
9	S3	1	Sibling	EBS	KRT5	E477K	Normal	Normal
8	S4	1	Sibling	EBS-DM	KRT14	R125C	Normal	Normal
6	S5	1	Sibling	EBS-DM	KRT5	I183F	Normal	Normal
6	S6	2	Sibling	EBS-DM	KRT5	I183F	Normal	**Normal**
10	S7	1	Father	EBS-WC	KRT5	I161S	Affected	Ltfu
16	S8	1	Mother	EBS-DM	KRT5	dell183	Normal	**Normal**

[a]Corresponds to the patient number in Table I.
[b]Refers to relationship of the previously affected family member with the fetus.
[c]EBS, unidentified subtype; EBS-DM, EBS Dowling–Meara; EBS-WC, EBS Weber–Cockayne.
[d]Two cases in bold have not been previously reported. The other cases were reported by Pfendner *et al* (2003).
Ltfu, lost to follow-up.

eight pregnancies tested, six were shown to have normal genotype, whereas two (S1 and S7) were diagnosed to have recurrence of dominant, paternally inherited disease. These predictions were confirmed in six cases by the birth of a healthy child. In case of the affected fetus in test S1, the birth of an affected fetus was confirmed by clinical observations, whereas the case in test S7 was lost to follow-up. In general, the most common reason given for the prenatal test was the knowledge to prepare for an affected child. Thus, prenatal testing in EBS is primarily used for counseling purposes rather than prevention.

Collectively, our data confirm that patients with an EBS phenotype harbor a spectrum of mutations in the KRT5 or KRT14 genes. Our results suggest a high rate of *de novo* mutations, as the corresponding mutations were absent in the parents' peripheral blood cell DNA and/or there was no family history of blistering diseases. Finally, we demonstrate the feasibility of prenatal diagnosis for EBS in families at risk for recurrence of the disease with phenotype of such severity that prenatal testing is warranted in preparation for the birth of an affected child.

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